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SIMPLIFIED APPARATUS AND TECHNIQUE FOR THE ELECTROMETRIC DETERMINATION OF HYDROGEN ION CONCENTRATION IN MILK AND OTHER BIOLOGICAL LIQUIDS

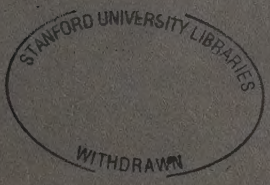
A THESIS

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF CORNELL UNIVERSITY FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

BY

ARTHUR JOHN RIDER



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FRANK E. RICE AND ARTHUR J. RIDER

A great variety of forms of apparatus have been recommended for the determination of hydrogen ion (H^+) concentration. Many of these are more elaborate than is necessary for ordinary purposes, and the procedures recommended for making the measurement are often rendered tedious with unimportant precautions. This is due partly to the fact that the influence of various factors on the accuracy and speed of the determination have not generally been known, very few systematic studies ever having been made from that point of view.

For purposes of convenience the apparatus for determining H^+ concentration can be considered essentially as of two parts. One includes the standard cell, galvanometer, slide wire resistance, source of current, and all wiring connections. This part of the potentiometer system is fairly well standardized, and the forms offered by the various manufacturers differ only in slight details. The assemblage and manipulation of this part of the system is so simple that there is practically no chance for error. The other part of the equipment, however — the glass parts, including the calomel cell, the hydrogen electrode cell, and the connections — is very much more sensitive. It is here that manipulation is extremely important. While the potentiometer system has been in common use for a long time, it is only comparatively recently that it has been very widely used for the measurement of H^+ concentration; consequently, it is the electrode vessels and appurtenances which are less standardized and upon which less comparative work has been done.

When the writers started H^+ concentration work, a number of forms of apparatus were procured and the procedures recommended by various investigators were tried. So much difficulty was found, and, on the whole, such poor results were obtained, that it was concluded a critical study should be made of the various recommendations of previous investigators as to apparatus and means of measuring H^+ concentration. Gradually the apparatus and the procedure described in this paper were evolved, and they have for some time proved satisfactory in the hands of many operators.

PURIFICATION OF THE MATERIALS

The method suggested by Moseley and Myers (1918) was generally used for the preparation of pure water. Mercury was purified by redistilling *in vacuo* several times. The method used for preparing pure calomel was that recommended by Clark and Lubs (1916). The

product was preserved in a dark place and kept covered with 0.1 N KCl. The purest KCl obtainable was recrystallized several times for use.

As a plentiful supply of hydrogen gas is necessary, particularly for the apparatus here to be described, it was found best to make use of cylinders containing the gas compressed. No doubt the purity of the gas will vary with the method of its original manufacture. The particular consignment which was used in this experimental work was found to be good; identical results were obtained whether or not the gas was washed before it reached the hydrogen electrode vessel. However, washing the gas is very simple, and it is probably wise to always take the precaution. In this work the gas was passed from the cylinder through successive wash bottles containing, respectively, alkaline pyrogallol, dilute sulfuric acid, mercuric chloride solution, and water, and finally through a calcium chloride tube containing dry cotton.

THE CALOMEL CELL

The essentials of the calomel cell are: a wire in contact with the mercury layer, leading outside; above the mercury, a standard KCl solution which is saturated with HgCl; then an arrangement, usually a siphon tube, to make contact between the KCl-HgCl solution and the remainder of the chain.

Cells containing normal, tenth normal, and saturated KCl are in common use. The tenth normal electrode is perhaps employed most generally; for one reason, among others, because the potential changes less with temperature. That electrode is the one used in this work.

The form of the vessel for the calomel electrode is unimportant. Most of those that are on the market are suitable, though it was found desirable to have a stopcock in the siphon tube such as is provided in the Clark calomel cell. The cell should be closed, in order to prevent changes in concentration of KCl which might result from evaporation.

The Clark cell and the Kelly cell obtained from the manufacturer were tested, together with a cell which was prepared in this laboratory. The last-named is illustrated in figure 1(A). It is simple in construction and was found to give results comparable with those obtained with the other two cells. In preparing this cell, a short piece of platinum wire is soldered to the end of copper wire. This is thrust through a glass tube until the platinum protrudes at one end, when that end is sealed in a flame. The tube is supported by a rubber stopper in a wide-mouth bottle of from 100 to 200 cubic centimeters capacity. In the apparatus used in this work, *a* and *b* were glass stopcocks, while *c* was a rubber connection with a spring pinchcock; but it would be possible at all these points to use rubber connections with pinchcocks.

In preparing the calomel cell, the vessel is carefully cleaned and dried. Mercury is placed in the bottom so that the wire will be covered. A paste is made up of the purified calomel with a little mercury moistened with 0.1 N KCl solution. This is run in over the mercury until there is a layer about five millimeters thick. Then there is added

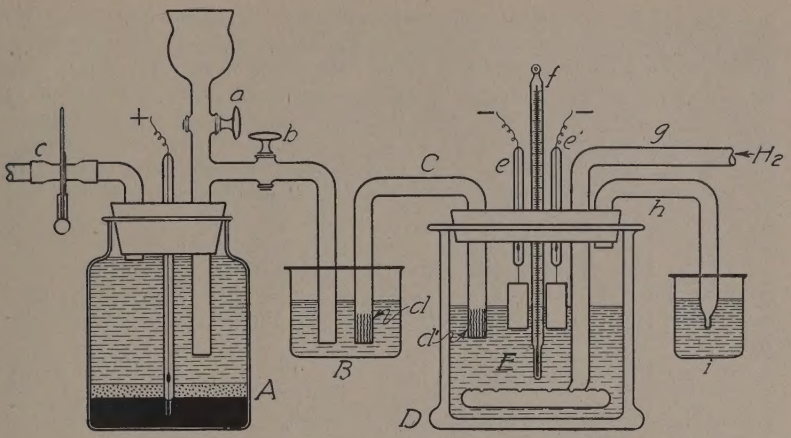

FIG. 1. APPARATUS FOR DETERMINATION OF HYDROGEN ION CONCENTRATION

0.1 N KCl solution which has been previously saturated with calomel. Care must be taken throughout this operation that the mercury layer is not disturbed, so that no drop of moisture will be left between the platinum wire and the mercury. A good contact at this point is necessary.

The cell should not be used for about two days after preparing, in order to give opportunity for equilibrium to become established.

The upper part of the vessel and the siphon tube should be occasionally flushed out, fresh KCl solution previously saturated with calomel being used. This is to prevent the interior of the cell from becoming contaminated through diffusion from the outside. It may be accomplished by filling at *a* and opening *b* and *c* for drainage.

The stopcock *b* at the siphon tube is ungreased and is kept closed during readings. This will prevent movement of the liquid through siphoning action, and also lessen the chance of contamination through this tube from the outside. At the same time, sufficient contact is made through the liquid film standing around the stopcock for a free flow of electric current.

THE HYDROGEN ELECTRODE

A hydrogen electrode consists of a sheet of metal, coated uniformly with spongy platinum, palladium, or iridium, which is suitable for absorbing hydrogen gas at its surface. Some workers have used glass on which, by a special process, platinum is deposited.

Gold has been recommended by some investigators for the preparation of electrodes, it being stated that with this metal a perfect saturation of hydrogen is more readily obtained, and, as a result, equilibrium comes more quickly. Both gold and platinum were tried in this work,

but the latter was found the more satisfactory. Two gold and two platinum electrodes were platinized similarly and H^+ concentration measurements made in 0.001 N HCl. Readings were taken from time to time until equilibrium was reached. Fourteen determinations were made with gold electrodes, and the average time required to reach equilibrium was found to be 12.5 minutes. On the same solution twenty-four determinations were made with platinum electrodes, with an average time to reach equilibrium of 7.3 minutes. It is seen that equilibrium was reached less rapidly by the use of gold electrodes than with platinum. The former has the disadvantage also that aqua regia cannot be used in cleaning. This reagent is particularly efficient in cleaning platinum black from electrodes. For these reasons the gold electrodes were not used in this work, but platinum was used exclusively.

The electrodes are prepared as follows: A piece of platinum foil is cut, about 1 to 2 centimeters square. Platinum wire is welded to the foil by laying the wire and the foil in position on a block of Alberene Stone or any stone that can conduct the heat away but slowly. A very small flame from a blast lamp is directed at the point to be welded, and when the platinum is at red heat a sharp blow with a light hammer is sufficient to make the union. The platinum wire should be from 10 to 15 centimeters in length, or an economy may be effected by using a piece of wire from 2 to 3 centimeters long and soldering to this a piece of copper wire. The wire is then pushed into a glass tube so that only platinum protrudes at the foil end, and this end is sealed in a gas flame.

The surface of the platinum is cleaned by immersing it in aqua regia or chromic-acid mixture and then rinsing it with water. Platinum black is deposited on the foil by making it cathode in a 3-per cent solution of chlorplatinic acid containing a trace of lead acetate — the lead acetate causing the platinum black film to adhere better than it would otherwise. Somewhat better results were noted when at intervals the direction of the current was reversed, thus having the foil as anode. In this way two electrodes can be platinized together.

When the surface of the platinum appears to be well covered with platinum black, it is removed from the solution and washed.

The size and shape of the platinum foil have been found not to influence the results, with one exception. An electrode consisting only of a small piece of platinum wire protruding from the support, similar to that described by Bovie (1915), at times gave results at variance with those from electrodes of larger surface exposure. There is one particular advantage in using the larger electrodes, in that, because of the size, the cell offers less resistance to the passage of current, and the whole apparatus is in consequence more sensitive.

Electrodes should not be allowed to become dry after preparation for use, but should be kept immersed in water or weak sulfuric acid solution.

Electrodes that have been used in fluids containing fats and proteins soon become useless. In some cases they can be regenerated by soaking in chromic-acid cleaning mixture, though frequently it is necessary to

clean them entirely and replatinize. Washing in warm aqua regia will remove the platinum black coating, after which the electrodes are ready for replatinizing.

In preliminary work some use was made of the electrode vessel designed by Clark (1915) and electrodes of the type suggested by Hildebrand (1913). A modification of the Hildebrand electrode was also prepared, with the tube supporting the platinum foil made to slide up and down in the outer shell. It was then possible to adjust the position of the foil so that there would be a maximum exposure of the foil to both the hydrogen gas and the solution.

It has been found in this work that it is very desirable to make readings from time to time for the calculation of the H^+ concentration, in order to be certain that the equilibrium point has been reached. The time required for obtaining equilibrium varies with the type of solution, and in some cases is very long. With the Clark vessel it is impossible to judge, except by making a number of separate determinations, when the equilibrium point is reached, since after one reading is made it is necessary to empty the apparatus and begin again. This is the chief objection to this cell.

In using electrodes of the Hildebrand type it was found that equilibrium was reached extremely slowly; in some samples of milk, bubbling hydrogen through the liquid as long as forty-five minutes was necessary before a constant reading could be obtained. This was believed to be due to the fact that with this type of electrode the surface of the liquid is in contact with air. Any type of electrode vessel which is so enclosed as to maintain an atmosphere of hydrogen over the liquid would eliminate this objection.

FACTORS INFLUENCING THE RAPIDITY WITH WHICH EQUILIBRIUM IN READINGS CAN BE REACHED

The literature on H^+ concentration determinations, and also laboratory experience, emphasize the desirability of finding an apparatus and a method which will bring about certain equilibrium with the least possible delay. Some investigators have reported that even several days were necessary at times for obtaining constant results. The most important points in this regard relate to the hydrogen electrodes and the design of the vessel. The calomel electrode, from a few days after preparation, remains constant for months. In some cases, after equilibrium has been reached a slight drift in potential is noticed, which may be due to diffusion at the surfaces between the solutions of different concentrations in the chain; but this can be counteracted by providing fresh surfaces each time a reading is to be taken, and finishing each measurement as rapidly as possible. The only other cause for variation in potentials pertains to the hydrogen electrode and the liquid bathing it. Correct potential is not obtained at this point until time has been given for the following general conditions to be reached: pure hydrogen gas exclusively must be adsorbed on the surface of the spongy platinum;

all foreign gases, especially oxygen, must be swept out of the liquid or reduced by the hydrogen at the surface of the platinum; substances capable of taking up hydrogen must be saturated with it; time must be given for diffusion of the liquid into the pores of the spongy metal.

A preliminary study was made of these factors by employing Hildebrand electrodes in various ways. Each condition and method of manipulation which was thought to affect the time of reaching equilibrium was taken up, with the end in view of finding those which would reduce the time to a minimum. The results are given in the following paragraphs.

Effect of depositing hydrogen on the platinized platinum foil by electrolysis

Loomis and Acree (1911) suggested placing the electrodes in a 2-per cent H_2SO_4 solution and making them cathode in a circuit. In this way hydrogen is liberated at the platinum black surface and a considerable adsorption of the gas takes place, with the result that less time is necessary for saturation of the electrode later, when it is immersed in the solution, and correspondingly less time should be consumed in reaching equilibrium of readings.

On determining the hydrogen ion concentration of 0.001 N HCl, four determinations were made, electrodes that had had hydrogen plated out on them, as just described, being used. Five, six, ten, and fifteen minutes, respectively, were required to obtain equilibrium in readings. Four determinations with electrodes not so treated required twenty, twenty, forty, and forty minutes, respectively.

Effect of passing gaseous hydrogen over the electrode

Robertson (1907) recommended that in preparation of hydrogen electrodes they be immersed in water, and hydrogen gas bubbled over them for some time.

In some measurements which were made on 0.001 N HCl, all the electrodes were prepared in exactly the same way, except that some were treated by immersion in this solution and bubbling hydrogen over them for a considerable period of time. An average of eight determinations when the electrodes were not so treated, showed that 10.9 minutes was required to reach equilibrium, and the average of ten determinations for those treated was 5.2 minutes.

These experiments show distinctly that the time required in reaching equilibrium of readings is due partly to the slowness with which the electrode itself is saturated with hydrogen, and that time is saved if they are given these preliminary treatments.

Effect of previously saturating the solution with hydrogen

As was pointed out by Konikoff (1913), oxygen or oxidizing substances in the solution are detrimental to the measurement of H^+ con-

centration because of a reducing action taking place at the surface of the electrode. Also, the presence of any gas other than hydrogen lowers the hydrogen pressure, and as a result an incorrect potential is observed. Because of this, it is important that any foreign gases be swept out and that any oxidizing substances be reduced, before accurate results can be obtained.

In making sixteen determinations on 0.001 N HCl, in half of which the samples had been previously treated by bubbling hydrogen gas through them, it was found that on the average 6.3 minutes was required for the samples which had been so saturated and 15 minutes was required for those which had not, all other conditions of the determinations being the same.

It is here seen that not only is saturation of the electrode necessary before equilibrium can be reached, but also saturation of the solution with hydrogen.

Effect of exposing the electrode alternately to the atmosphere of hydrogen and to the liquid

It has been suggested by Clark (1915) that in order to reach equilibrium rapidly the H^+ cell should be so built that the entire electrode is bathed alternately with hydrogen gas and with liquid. This has been made a special point also in the construction of the Bunker (1920) electrode.

In experimenting with the Hildebrand electrode and 0.001 N HCl, thirty-two determinations were made in which the electrode was raised by hand into the hydrogen and lowered into the liquid alternately until equilibrium was reached; it was found that, as an average, 8.4 minutes was required for equilibrium. Thirty-two determinations were made also with similar electrodes without attempting to regulate the rise and fall of liquid over the electrode. In some of these cases the time required for equilibrium was less than the above-named average; however, the average of all determinations was 12.3 minutes.

It is seen that a definite advantage is gained by so handling the Hildebrand electrode that it is bathed alternately with liquid and gas. In most of the electrodes designed to accomplish this result, a mechanical shaking or rocking device is used. However, it is believed that the same result can be obtained much more easily by admitting a rapid stream of hydrogen into the liquid immediately below the electrodes. This scheme is used in the hydrogen electrode vessel eventually prepared for this work, which is described later. Similar results are obtained with the electrode vessel designed by Loomis and Acree (1911).

Effect of shaking the electrode vessel

The use of electrode vessels equipped with shaking devices has been common. It has been believed that as a result of shaking there is a more rapid interchange between the solution, the gaseous hydrogen, and the electrode, also that any molecular or combined oxygen in the solu-

tion is more quickly brought into contact with the electrode and reduced.

Throughout the course of these experiments it has been observed that it is of decided advantage to keep the liquid in vigorous agitation, either by shaking the electrode vessel or by the rapid bubbling of hydrogen through the solution. So far as could be seen, neither of these methods has any particular advantage over the other in establishing equilibrium rapidly. Since a mechanical device is necessary if shaking of the electrode vessel is to be a part of the procedure, it was omitted in the apparatus which was finally adopted, and agitation was brought about by a very rapid bubbling of hydrogen gas entering at the bottom of the liquid.

AN IMPROVED HYDROGEN ELECTRODE CELL

Individual hydrogen electrodes occasionally give incorrect results, sometimes through intimate adsorption of impurities, commonly called *poisoning*. Also, particularly after being used in biological liquids, the surfaces may become coated with fats, proteins, or other organic substances, so that abnormal voltages are yielded. It is therefore desirable to use more than one electrode as a check on results.

In the cell eventually assembled for use in this laboratory, it is possible to include as many as six electrodes, and merely by switching from one to another, readings can be taken on all without delay.

This cell (figure 1, *D*) consists of a glass cylinder about 7 centimeters in diameter and 10 centimeters deep, fitted with a rubber stopper through which are holes admitting the electrodes (*e* and *e'*), a thermometer (*f*), a hydrogen inlet tube (*g*), a hydrogen outlet tube (*h*), and a siphon tube (*C*) connecting the body of the liquid with the reservoir (*B*), which in turn is in contact with the liquid in the calomel electrode vessel.

The hydrogen inlet tube terminates in a horizontal ring which is perforated to give a large number of openings so as to distribute the flow of hydrogen through the body of the liquid. Thus a rapid and complete saturation of both liquid and electrodes is effected.

The hydrogen outlet tube may dip into a reservoir of water (*i*), thus acting as a water seal and securing an atmosphere of hydrogen throughout the interior of the cell by preventing an access of air. However, as long as the hydrogen is flowing freely there is no opportunity for the entrance of air, and the use of the water seal is not then necessary.

Assembling the apparatus

Making contact between the mercury and the hydrogen electrodes

Calomel cells and hydrogen electrode cells must be joined by means of a solution which is highly conducting, since for maximum sensitivity there should be offered no more resistance than is necessary to

the passage of the current. A saturated solution of KCl is ordinarily employed, and that was used in this work. The siphon tube *C* was filled with this solution, and the reservoir *B* with 0.1 N KCl. The tube *C* is of about five millimeters internal diameter and is plugged at the ends with small rolls of filter paper, which prevent a loss of the liquid when the tube is raised, and also lessen diffusion. This tube should not fit so tightly in the rubber stopper but that it can be raised and lowered easily.

This assemblage is designed to reduce contact potential to a minimum, which is a possible source of error depending on the fact that a potential is set up between two liquids of dissimilar concentration at their juncture. For example, an electromotive force is set up between the saturated KCl and 0.1 N KCl solutions, and also, on the other side of the chain, between saturated KCl and the liquid in the hydrogen cell. For extremely accurate work this is taken into account and calculated by the classic method of Bjerrum (1905). However, it has been found by the same investigator that this source of error is reduced to the vanishing point by the use of saturated KCl as previously described, for the differences of potential at the liquid junctions at the two ends of the saturated KCl solution are about the same. Since the potential differences are in opposite directions, they tend to equalize each other.

Equipotential shielding of the apparatus

There were periods during the investigation when it seemed impossible to obtain results of any regularity, this difficulty seeming to come chiefly in damp weather. On the assumption that the trouble was due to stray electric currents, the suggestions of White (1914) were followed to insure protection against any such effects. The desk top was covered with a large sheet of aluminum. Then under each piece of apparatus was placed a piece of glass. Thus each instrument was insulated from every other one except through the proper connections, and the whole system rested upon an equipotential base which shielded it from stray currents. No further trouble of this nature was encountered. As a precaution, shielding should never be omitted.

Making the determination of hydrogen ion concentration

After the sample on which determination is to be made is placed in the hydrogen electrode vessel, the electrodes should be adjusted so that the foils are about half submerged. Careful inspection should be made of the siphon tube *C* and the tube leading out of the calomel cell, to make sure that no air bubbles are present, in order that the contact may be perfect throughout the chain.

The proper connections are made from the mercury and the platinum poles to the potentiometer. The use of knife switches makes convenient the change from one hydrogen electrode to another. A rapid flow of hydrogen is maintained throughout.

The siphon tube *C* should be raised except while the readings are being made. After a few minutes the first readings are taken, and readings are continued from time to time, first with one electrode and then with the other, until there is no longer any change. The hydrogen need not be shut off while the measurements are being made. The time at which equilibrium is reached depends upon many things, particularly the nature of the sample. With milk, twenty minutes is sufficient, though it is possible that there are other biological liquids which require a longer time.

Generally, no trouble was experienced from frothing, either with raw milk or with milk reconstituted from evaporated and condensed milk. Powdered milk from a large number of manufacturers was studied, and in but one instance was any difficulty experienced in this regard, and it was not then impossible to obtain results. It may be noted that the use of octyl alcohol has been found satisfactory by Schmidt (1916) on such occasions.

In addition to being able to check hydrogen electrodes one against the other, it is also nearly as desirable to have results from more than one calomel cell. Any number of calomel cells can be prepared, two or more can at the same time dip into reservoir *B*, and readings can be taken from all for comparison.

Readings are made on the potentiometer in volts or millivolts, and it remains then to calculate H^+ concentration from these results. Tables given by Schmidt and Hoagland (1919) were made use of in this work.

Correction for temperature

Most methods of calculation and tables are designed for measurements to be made at 25° C. Many investigators enclose the apparatus in an air bath or a bath of liquid, and thus hold the temperature constant at 25° or any other point desired. The use of constant-temperature rooms adds a considerable expense and trouble to the manipulation, and the immersion of the whole apparatus in a liquid is liable to cause errors through current leakages. On the other hand, it has been found in this work that the temperature correction methods suggested by Schmidt and Hoagland do not give good results.

The method which was finally adopted and which yielded satisfactory results was to hold at 25° C. only the sample on which the H^+ concentration measurement is to be made. This can easily be done by immersing the hydrogen electrode vessel in a water bath. By observing the thermometer which dips into the sample, the temperature of the sample can be brought to the desired point.

While the hydrogen electrode is very sensitive to a variation of a single degree of temperature, the potential of the calomel cell changes but little with small variations, and it was believed unnecessary to maintain it at a constant temperature. However, it is best not to allow the temperature of the calomel cell to vary too widely from 25°. The room should be not below 20° C.

EFFECT ON DETERMINATIONS OF HAVING ELECTRODES WHOLLY OR PARTLY IMMERSED IN THE LIQUID

Clark and Lubs (1916) make a point of the fact that readings were taken with the electrode wholly immersed in the liquid. McClendon and Sharp (1919) state: "If the solution to be tested is sufficiently viscous to support a layer of the solution on the platinized electrode (as in the case of blood plasma), it is not necessary to take the reading with the electrode totally immersed."

Some experiments were made on milk, measuring the potential with the electrodes wholly immersed and with the electrodes only half immersed, and it was found that if the electrodes were small — less than about one centimeter square — there was generally a difference of from 1 to 3 millivolts, the electrodes wholly immersed giving the higher values. With very large electrodes no differences were noted. At least one square centimeter of foil should be immersed in the liquid at the time of making the reading.

It will be remembered that in the manipulation of the H^+ apparatus previously described, it is recommended that the readings be taken with the full flow of hydrogen passing through. Even though the electrodes are only about half submerged when the liquid is at rest, the continual bubbling of the gas passing through the liquid below the electrodes keeps the entire surface of the foil well covered, and the same effect is produced as though the entire electrodes were really beneath the surface.

RESULTS OBTAINED WITH THE USE OF THE HYDROGEN ELECTRODE EQUIPMENT HERE DESCRIBED

On standard buffer solution

Solutions of known H^+ concentration are often used in checking the behavior of H^+ concentration apparatus. A standard solution of acid potassium phthalate was made up according to Clark and Lubs (1916), which has a P(H) value of 6.711. The following results were obtained with six hydrogen electrodes, running two at a time, five determinations being made with each pair:

Determination	Electrode					
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
1	6.696	6.696	6.708	6.708	6.708	6.708
2	6.707	6.707	6.704	6.704	6.704	6.704
3	6.712	6.712	6.702	6.702	6.722	6.722
4	6.712	6.712	6.708	6.708	6.698	6.698
5	6.717	6.717	6.710	6.710	6.713	6.713
Average	6.709	6.709	6.706	6.706	6.709	6.709

These results, obtained by the use of the apparatus here described, show the variations (1) between different electrodes, (2) in making

different determinations on the same solution, and (3) between the true values and those obtained by the apparatus.

On samples of raw milk and manufactured milk reconstituted with water

Determinations totaling 459 were made on samples of raw milk, also on evaporated and powdered milk reconstituted with water according to the manufacturers' directions. In each determination, readings were taken using hydrogen electrodes in pairs. In 444 cases identical results were obtained with the two electrodes. In the remaining 15 cases the differences varied from 0.0004 to 0.0010 volt, which represented a variation in P(H) value of about 0.007 to 0.017. The samples ranged from 6.543 to 7.022.

EFFECT OF CARBON DIOXIDE UPON HYDROGEN ION CONCENTRATION

As has been pointed out by Clark (1920), in solutions with P(H) values above 5 the presence of carbon dioxide becomes of more and more importance. In the determination of H^+ concentration by use of the apparatus here described, this gas would of course be driven out of solution. If carbon dioxide is present in a nearly neutral liquid, and it is desired to measure acidity due to all factors including this (as in the case of blood, mineral water, and so on), then the apparatus here described should not be used. Under such conditions an electrode vessel of the closed type should be employed.

Except in a few cases such as in blood studies, however, carbon dioxide is not a part of the intricate physical chemical arrangement of the various soluble constituents which are important in physiological investigations. A soil solution, for instance, with a low P(H) value due to carbon dioxide, might be an extremely fertile soil, since carbon dioxide has a highly solvent action on the mineral matter in soil; on the other hand, an equally low P(H) value due to some fixed soluble acid might be extremely detrimental. Carbon dioxide is present in considerable quantity in freshly drawn milk, and it perhaps influences the H^+ concentration slightly. However, acidity due to this gas cannot be of any importance in manufacturing operations, since the gas would soon be driven out, nor is it likely to be worthy of consideration in market milk. Also, in any purely scientific investigation of milk wherein H^+ concentration determinations are studied, whatever slight effect the presence of this gas may have should be disregarded.

The apparatus for H^+ concentration determination here described is therefore recommended for experimentation on all biological liquids except in those few cases in which carbon dioxide is of prime importance as such.

HYDROGEN ION CONCENTRATION DETERMINATION WHEN THE AVAILABLE AMOUNT OF SAMPLE IS SMALL

There are occasions in biological work when only a small amount of sample can be obtained. In such cases it is necessary to use a hydrogen

electrode cell of small dimensions. While cells used in this work contained as many as six hydrogen electrodes, and all parts were of liberal proportions, by which means it was believed that the most nearly accurate results could be obtained, it would be posible to reduce the size considerably in every way and use only one or two hydrogen electrodes. By careful manipulation of such an apparatus entirely reliable results can be obtained.

ADAPTATION OF THE APPARATUS FOR ELECTRO-TITRATION

Generally in electro-titration, results of any considerable degree of accuracy are not obtained. Vessels are used which are open to the air, and the designs are such that a satisfactory saturation of the hydrogen electrode cannot be carried out.

By merely providing another hole in the stopper of the apparatus here used, and the introduction of a burette, electro-titrations can be readily made. Since the hydrogen gas is admitted rapidly at the lower layers of the liquid, there is sufficient agitation so that it is not necessary to provide a mechanical stirrer.

SUMMARY

An investigation has been made of the influence exerted by certain variations in procedure upon the speed and accuracy of the determination of H^+ concentration. With the knowledge thus gained, an apparatus has been designed which is simple of construction and easy and rapid of manipulation.

The apparatus is designed so that several hydrogen electrodes can be checked against one another, as well as comparisons made between different calomel cells. One reading after another can be made on a given portion of liquid, thus determining the equilibrium point without changing samples.

A simple yet accurate means of temperature control is suggested.

This apparatus is well adapted for use in student laboratories, as well as in research work; and merely with the introduction of a burette, electro-titrations can be made more nearly accurately than by the apparatus ordinarily employed for that purpose

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NOV 18

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NOV 18 '31